March 21, 1971

Dr. Peter Vogt
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Dear Peter,

Enclosed with this letter you will find copies of papers recently written by us and by Malcolm Martin's group at NIH. As you will see from these preprints, both mouse and chick virus polymerases appear to synthesize double-stranded DNA, which is principally of low complexity - representing less than 5% (our interpretation based on Fd RF and λ standards) or about 20% (their value based on an SV 40 standard) of the viral 70S RNA genome. There is also at least one additional population of double-stranded DNA molecules produced, this population being of greater complexity (12% of the genome in our hands, perhaps 100% in Martin's). Measuring the rate of reassociation of the large population (RR fraction, for "rapidly reassociating") in the presence of unlabeled cell DNA's, we find about 20 copies of the RR sequences in both chick embryo DNA and in DNA extracted from SR-RSV induced tumors. Salmon sperm DNA (our viscosity control) has no copies. As you can see from their paper, Gelb et al. also find multiple copiesoof their rapidly reassociating fraction in normal and transformed (including "non-producing") rat and mouse cells, and in African green monkey kidney cells. We are about th look for the sequences in HeLa cells and may look at other animal species as well. In addition, we are trying to determine the chromosomal location of the RR sequences by using chick DNA extracted from chromosome populations fractionated in sucrose on the basis of size. Because the RR sequences represent a small fraction of the viral genetic information, we are also using single-stranded DNA product (prepared from RNA:DNA hybrids and presumably containing all the viral sequences) as a probe for viral information in normal and transformed cells. Preliminary experiments in our lab and Martin's indicate that the transformed DNA does contain more viral sequences than normal cells.

Of course, at this point the significance of the RR copies in normal chick cells is uncertain - we do not yet have any real

idea what the gene products of the RR DNA might be. But we are sure you will agree that it would be of particular interest to know whether c/o' cells, which appear to lack viral antigens and cryptic RAV-60, are also lacking the RR sequences, It is possible, of course, that we are not yet using the best RR probe, since ours is synthesized by SR-RSV polymerase; we would be interested in your opinion about the feasibility of preparing sufficient amounts of RAV 60 to use for RR synthesis.

We consider thebbiochemical experiments we are suggesting with c/o' cells to be principally attempts to extent your biological findings and Hanafusa's from another point of view. We are very pleased that you are willing to assist us in these experiments by sending us the cells and hope we will soon be in touch about questions or results.

Yours,

Harold E. Varmus, M.D. Warren Levinson, M.D., Ph.D.

J. Michael Bishop, M.D.
Department of Microbiology

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